fund of knowledge; but like reproduction by division, its products may be new individuals, in new places, but remarkably similar to what we already have. The interrelation of "unknowns" points to a need for central direction and coordination of all these research efforts. Like water itself, our major water problems tend to resist partitioning, and without coordination of effort they may well remain unsolved.

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Biosynthesis of Unsaturated Fatty Acids in Microorganisms

Structures and biosynthetic pathways are compared and related to physiological properties of the organisms.

Joseph Erwin and Konrad Bloch

The role that lipids play either as structural components of the cell or in metabolic processes is largely a matter of conjecture. That triglycerides serve as mobile carbon reserves is undisputed, but no definite functions can as yet be assigned to the numerous phospholipids, glycolipids, sphingolipids, cerebrosides, and their structural variants. There is essentially no information on the metabolic consequences arising from structural modifications of a given lipid molecule—for example, when the nitrogenous base of a glycerophosphatide is replaced by another nitrogenous base, by an amino acid, or by a nitrogen-free polyol. Similarly, it is recognized that the length and the degree of unsaturation of a fatty acid residue in ester linkage profoundly affects the properties of lipid molecules, but what these effects are in terms of biological function is only poorly understood. It is evident, at any rate, that the basic structures of the lipids are extremely flexible and allow for a wide variety of modifications. The composition of a lipid molecule in all its details is probably determined not only genetically but also by environment. Thus it is known that nutrition, temperature, and the gaseous atmosphere, as well as other external factors, can modify the lipid pattern of organisms. It is perhaps this flexibility and the ready adjustment of lipid structures to a changing external environment that promises to provide some deeper insight into the structure-function relationships of the lipids.

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As we have pointed out elsewhere (*1*), comparative studies are especially useful for assessing the significance of structural modifications in relation to function. There are marked differences in structure and composition between the lipids of various species, between tissues of the same organism, and also between the cytoplasmic constituents of a given cell. By examining a wide variety of cell types and by making the selection on the basis of distinctive morphological and physiological characteristics, one may hope to detect some systematic pattern in the bewildering diversity of lipid structures.

In order to illustrate the potentialities of the comparative approach, we present in this article some of our results on pathways of biosynthesis of mono-unsaturated and polyunsaturated fatty acids in various groups of microorganisms. Secondly, as an example of environmental effects, we discuss poly-unsaturated-fatty-acid patterns in a number of photosynthetic organisms and the relation of these patterns to phototrophic and heterotrophic forms of metabolism.

Biosynthesis and Distribution of Mono-unsaturated Fatty Acids

Most cell constituents arise by a single pathway of synthesis, and the chemical reactions of this pathway are ordinarily the same in all biological systems. However, as has recently been pointed out, there are several exceptions to this principle of biochemical unity (*1*).

Thus, two chemically distinct pathways have been recognized for mono-unsaturated fatty acids (*2*), and evidence is accumulating (*3*) that still another route may exist for the synthesis of compounds of this type (Table 1). In one of the two pathways (Table 1, pathway II) long-chain fatty acyl esters of coenzyme A are desaturated to the corresponding *cis*-Δ^9-monoenoic acids by enzymes which are particle-bound. This mechanism requires oxygen and occurs in a great variety of phylogenetic groups—in yeast (*4*), in animal tissues (*5*), in protozoans (*6*), in some primitive algae (*7*), and in certain bacteria (*8*). The other pathway (Table 1, pathway I) differs in several respects. It is part of a soluble fatty acid synthetase system, it proceeds anaerobically, and its produces monoenoic acids by β-, γ-dehydration of medium-chain β-hydroxy acids and subsequent chain elongation of the resulting 3-enoates (*9*). This anaerobic mode of synthesizing unsaturated fatty acids is relatively rare. It is found in certain eubacteria but has not been detected in other microorganisms or in higher forms. In terms of distribution among organisms, the two mechanisms seem to be mutually exclusive; no organism has been found, so far, to contain the enzyme systems for both the aerobic and the anaerobic routes for the synthesis of unsaturated fatty acids.

It appears that a mechanism distinct from these two relatively well characterized pathways of biosynthesis operates in higher plants (*3*, *10*) and in certain photosynthetic protists (*11*). The nature of this third mechanism is obscure. The conclusion that it exists is based less on positive evidence than on the negative outcome of experiments made in an attempt to demonstrate, in plants, one or the other of the two known routes—oxidative desaturation or the anaerobic pathway.

Since the synthesis of cell constituents by multiple routes is relatively uncommon, it seemed of interest to ascertain whether a particular mode of synthesizing unsaturated fatty acids could be correlated with phylogenetically significant morphological, physiological, or biochemical characteristics. For this purpose our survey of organisms has been extended to additional species of bacteria and, in particular, to various groups of microorganisms which occupy intermediary phylogenetic positions between the bacteria on the one hand and the Metazoa and Metaphyta on the other. The organisms examined to date are listed in Table 2. They are arranged in three groups according to their mechanism for synthesizing unsaturated fatty acids. Groups I and III comprise relatively uniform classes of organisms, while group II covers a much wider spectrum, ranging from the bacteria to the Metazoa.

Table 1. Pathways for the synthesis of mono-unsaturated fatty acids.

Pathway I: Anaerobic

$$C_8 \xrightarrow{C_2} \Delta^3\text{-}C_{10} \xrightarrow{4C_2} \Delta^{11}\text{-}C_{18}$$

$$C_{10} \xrightarrow{C_2} \Delta^3\text{-}C_{12} \longrightarrow \Delta^9\text{-}C_{18}$$

*Pathway II: Oxidative desaturation**

$$C_{18} \xrightarrow{O_2} \Delta^9\text{-}C_{18}$$

$$C_{16} \xrightarrow{O_2} \Delta^9\text{-}C_{16}$$

Pathway III: "Plant" pathway

$$C_{18} \leftrightarrow \Delta^9\text{-}C_{18}$$

$$C_{16} \leftrightarrow \Delta^9\text{-}C_{18}$$

$$C_{12}(C_{14}) \xrightarrow{O_2} \Delta^9\text{-}C_{18}$$

* Exceptions to the Δ^9-specificity of oxidative desaturation are the Δ^5-C_{16} and Δ^5-C_{18} acids formed in *Bacillus megaterium* (*13*) and the Δ^{10}-C_{16} acid of *Mycobacterium phlei* (*12*).

Biosynthesis in anaerobic organisms is, of necessity, anaerobic. However, since group I ("anaerobic mechanism") includes not only anaerobic bacteria but also a facultative aerobe (*Escherichia coli*) and the obligately aerobic pseudomonads (*12*), it is clear that the organism's mode of energy metabolism does not determine whether the pathway used is aerobic desaturation or the "anaerobic pathway." On the basis of a more limited survey we had previously suggested that the association of pathway I with some bacterial groups and of pathway II with others may have some evolutionary significance and may provide a biochemical basis for the taxonomic distinction between eubacteria and actinomycetes (*2*). However, this generalization has now become untenable, since we have found the aerobic desaturation mechanisms to be present in representatives of three families of the order Eubacteriales—in *Corynebacterium diphtheriae*, in *Micrococcus lysodeikticus*, and in *Bacillus megaterium* (*13*). It is still true that the anaerobic mechanism is an exclusive property of certain eubacterial groups, but since aerobic desaturation of fatty acids occurs in several families of the order Eubacteriales, as well as in the actinomycetes, the mode of synthesis of unsaturated fatty acids is clearly not a valid marker for distinguishing the two bacterial groups. It is nevertheless tempting to assume that the existence of the two pathways has some evolutionary meaning in the sense that the anaerobic route is the more primitive of the two and that the advent of oxidative desaturation signifies an evolutionary advance, even though the morphological or physiological expressions of this advance are not yet apparent.

The aerobic, direct desaturation route to olefinic acids is the mechanism most frequently found, and it appears to be continuous throughout evolution from the Monera to the Protista and the Metazoa. On the other hand, evolutionary specialization terminating with the higher plants seems to have led to the loss of the oxidative desaturation pathway to monoenoic acids and to the appearance of a mechanism of still unknown nature (pathway III). The occurrence of a "plant" pathway distinct from pathways I and II is not yet

Table 2. Mechanisms of synthesis of mono-unsaturated fatty acids.*

Anaerobic, soluble	Oxidative desaturation, particulate or microsomal	Unknown (mitochondrial?)
Group I	Group II	Group III
1) True bacteria:	1) True bacteria:	1) Protista and algae:
Clostridia	*Micrococcus lysodeikticus*	Phytomonads
Lactobacilli	*Bacillus megaterium*	*Euglena gracilis* (light and dark)
Escherichia coli	*Corynebacterium* sp.	Green algae
Pseudomonads	2) Actinomycetes:	2) Metaphyta:
Photosynthetic bacteria	Mycobacteria	Avocado mesocarp†
	3) Algae and protista:	Castor bean leaves‡
	Cyanophyta, red algae	
	Chrysomonads	
	Astasia longa (euglenid)	
	Yeasts and fungi, *Penicillium* sp.	
	Amebae, ciliates	
	4) Metazoa:	
	Vertebrates	
	Invertebrates	

* The biosynthesis of unsaturated fatty acids has been studied at the enzyme level only in yeast, rat liver, *Mycobacterium phlei*, *Escherichia coli*, and extracts of avocado mesocarp. In all other cases the assignments to groups I, II, and III are made on the basis of in vivo experiments. Organisms are placed in column 1 if they synthesize unsaturated fatty acids from C^{14}-acetate anaerobically as well as in air; in column 2 if cells convert stearate or palmitate into octadecenoate or hexadecenoate, respectively; and in column 3 if the organisms fail to desaturate stearate or palmitate and require aerobic conditions for oleate synthesis. † See *3*. ‡ See *10*.

Table 3. Distribution of polyunsaturated fatty acids in major groups of protists grown on synthetic media. All the organisms listed contain palmitoleic, oleic, and linoleic acids. 0, Not present; ±, trace amounts; +, minor component; ++, major component; −, not determined.

Fatty acid	Yeasts and fungi	Algae			Protozoa			
		Blue-green	Red	Green	Ciliates	Amebae	Phytoflagellates	
							Green	Colorless
$\Delta^{4,7,10,13}$-C_{16}	0	0	0	++	0	0	++	0
$\Delta^{9,12,15}$-C_{18} (α-linolenate)	+	++	+	++	0	0	++	±
$\Delta^{6,9,12}$-C_{18} (γ-linolenate)	0*	0	0	0	++	0	+	++
$\Delta^{5,8,11,14}$-C_{20} (arachidonate)	0	0	−	0	0	+	±	++

* Except in *Phycomyces blakesleeanus* (*18*).

proved conclusively. However, the suggestive evidence is strong, since the properties of the oleate-synthesizing "plant" enzyme systems (Table 2, group III) distinctly differ from the properties of the enzymes involved in pathway I or pathway II. Thus, oleic acid synthesis in higher plants and in the organisms of group III is aerobic, but neither stearate nor palmitate serves as precursor. On the other hand, the carbon chains of myristate and laurate are elongated and, in some unknown manner, transformed to oleate by these organisms (*3, 10, 11*).

We have designated pathway III as the "plant" mechanism even though *some* photosynthetic organisms, particularly the more primitive ones, do not employ this mechanism. In photosynthetic bacteria the mechanism of synthesis of mono-unsaturated fatty acids is of the type found in anaerobic bacteria (pathway I); the blue-green (*7*) and red (*14*) algae do not belong to this group because in them oxidative desaturation (pathway II) is the mechanism of synthesis. Thus, neither photosynthesis per se (either of the primitive or of the advanced, oxygen-evolving type) nor the presence of chloroplasts can be correlated with the specialized "plant" pathway. On the other hand, it is perhaps significant that in some plant systems the mitochondria are the site of oleic acid synthesis (*3*), whereas pathway I is associated with soluble enzymes and pathway II with microsomal particles. Mitochondria are not present in bacteria or in either the blue-green or the red algae (*15*), hence the appearance of the "plant" pathway could conceivably signify the development of certain more highly differentiated, internal-membrane structures.

For the purpose of comparing biosynthetic and physiological properties, the phytoflagellates are of exceptional interest because they include strictly heterotrophic and strictly phototrophic groups as well as organisms having a "mixed" pattern of metabolism. For example, the chrysomonads are a relatively unspecialized physiological group of phytoflagellates, the capacities for photosynthesis, heterotrophic metabolism, and phagotrophy all residing in the same organisms (*16*). Significantly, the predominantly heterotrophic representatives of this group, *Ochromonas malhamensis* and *Poteriochromonas stipitata*, form oleic acid by direct desaturation of stearate (*14*). It is conceivable that the "plant" pathway also exists in these intermediary forms, but to establish this will be difficult as long as the means for detecting this pathway are indirect. Further along the evolutionary line toward the higher plants, the phytomonads (*Chlamydomonas* and *Polytoma*) and the euglenids (*Euglena gracilis*) are the most primitive of the green organisms in which the plant pathway is the only mechanism of synthesis—that is, the most primitive of the green organisms that fail to desaturate stearate to oleate. In *E. gracilis* this characteristic is found not only in cells raised under conditions that favor phototrophic growth but also in dark-adapted cells that lack chloroplasts (*14*). Similarly, in *Polytoma uvella*, a heterotrophic chlamydomonad that lacks chloroplasts, the "plant" pathway is the mechanism for synthesizing mono-unsaturated fatty acids. The conclusion to be drawn from the behavior of dark-adapted *Euglena* and of *Polytoma* is clearly that photosynthetic activity is not a prerequisite for the occurrence of the "plant" pathway, even though in higher plants oleate can be synthesized in chloroplasts (*3, 10*). The behavior of *Astasia longa* is anomalous. This naturally occurring phytoflagellate, which lacks chloroplasts and proplastids but is otherwise morphologically identical with *E. gracilis*, does synthesize oleate by desaturation of stearate (*14*). Thus it appears that the mutant-wild type relationship leads, in the case of *Chlamydomonas* and *Polytoma*, to a retention of the "plant" pathway, whereas in the case of *Euglena* and the morphologically similar *Astasia*, there is a reversion to, or reacquisition of, oxidative desaturation (pathway II).

Table 4. Major pathways for synthesis of polyunsaturated fatty acids.

Dienoic acids

1) Δ^9-$C_{18} \xrightarrow{O_2} \Delta^{9,12}$-$C_{18}$

2) Δ^9-$C_{18} \rightarrow \Delta^{6,9}$-$C_{18}$

α-Linolenate ("plant") pathway

$\Delta^{9,12}$-$C_{18} \rightarrow \Delta^{9,12,15}$-$C_{18}$

γ-Linolenate (animal) pathway

$\Delta^{9,12}$-$C_{18} \rightarrow \Delta^{6,9,12}$-$C_{18}$

$\Delta^{6,9,12}$-$C_{18} \xrightarrow{C_2} \Delta^{8,11,14}$-$C_{20} \rightarrow \Delta^{5,8,11,14}$-$C_{20}$

As we pointed out earlier, it is by no means proved that a separate "plant" pathway for the synthesis of mono-saturated fatty acids exists. If, in spite of the contrary present evidence, in plants oleate is synthesized from stearate by oxidative desaturation, then it is difficult to understand why in the organisms of group III (but not in those of group II) stearate is totally inactive as a precursor of oleate. To assume the existence of impermeable barriers or of noninteracting pools does not adequately explain the experimental findings.

Polyunsaturated Fatty Acids

Distribution. Though multiply unsaturated fatty acids occur in a bewildering variety of structural types, they can be assigned to two principal categories, one represented by α-linolenate and the other by γ-linolenate and arachidonate as prototypes. Linoleic and α-linolenic acids are the major polyunsaturated fatty acids of the green tissues of higher plants (*17*), while arachidonic acid and similar C_{20}- and C_{22}-polyunsaturated acids of the γ-linolenate type are characteristic lipid constituents of higher animals. Bacteria lack polyunsaturated fatty acids altogether, regardless of the type of mono-unsaturated acids which they synthesize (*12*).

As already stated, the protistan groups provide a special opportunity for comparing lipid patterns because in phylogenetic position the higher Protista are intermediate between the bacteria and the multicellular organisms and because their physiological activities are manifold. Table 3 shows, in a qualitative manner, the distribution of the major polyunsaturated fatty acid com-

ponents in a number of protists and the marked differences that exist between the various groups. That the synthesis of polyunsaturated acids occurred early in evolution can be inferred from the fact that the blue-green algae, though morphologically as primitive as the bacteria, contain linoleic and α-linolenic acids (*7*). So do several green algae and so does *Porphyridium cruentum*, the sole red algae examined, but *P. cruentum* under the chosen growth conditions (dim light) had a low content of α-linolenate (*14*). This relatively simple "plant" pattern of fatty acids is maintained in the yeasts and fungi, but in some yeasts, like *Saccharomyces*, the content of α-linolenate is usually rather low. Only one report (*18*) mentions the occurrence of γ-linolenate in fungi, and this occurrence is in the Phycomycetes, a relatively primitive group (*19*).

The lipids of Protozoa present a more varied picture. The typical "animal-like" protozoans, the amebae and the ciliates, synthesize fatty acids of the γ-linolenate type, and, like higher animals, they do not contain α-linolenic acid when cultivated on fat-free media. The ameba *Hartmannella rhysodes* produces significant amounts of arachidonic acid, while, in the ciliates, polyenoic acid synthesis terminates with γ-linolenic acid, the intermediate in the formation of arachidonate by vertebrates (*6*). Uniquely among animal cells the amebae and ciliates carry out the complete sequence of desaturations from oleate to γ-linolenate, including the conversion of oleate to linoleate. For this reason the "animal-like" protozoans, in contrast to the metazoans, are independent of an exogenous supply of "essential" fatty acids. The polyenoic-acid pattern in vertebrates is clearly related to that in the ciliates and the amebae. One need only assume that one of the required biosynthetic steps toward synthesis of "essential" fatty acids, the $\Delta^9 \rightarrow \Delta^{9,12}$ desaturation, was deleted during the evolutionary process.

In contrast to the animal-like protists and the primitive algae, phytoflagellates contain, in the same organism, the multiply unsaturated fatty acids characteristic of animals and those characteristic of plants. Thus, in the chrysomonads *Ochromonas danica* (*20*), *O. malhamensis* (*14*), and *Poteriochromonas stipitata* (*14*) and in the euglenids one finds considerable amounts of arachidonic acid and γ-linolenic acid in addition to linoleic and α-linolenic acids. In *Chlamydomonas*, too, both α- and γ-linolenic acids are present.

An unusual compound encountered so far only in photosynthetic phytomonads, in the euglenids, and in several green algae is a C_{16}-polyunsaturated acid, identified by Klenk and Knipprath as a 4,7,10,13-tetraene (*21*). This acid is a major lipid component in *Scenedesmus obliquus* and also in *Euglena gracilis* (*22*), provided the phytoflagellate is grown in the light.

The fact that the two types of polyenoic acids occur as constituents of different classes of lipids is mentioned here only briefly, although this is a very interesting subject in itself. The main point to be made is that linoleate, γ-linoleate, arachidonate, and the C_{20}-polyenic acids of similar structure are found predominantly in phospholipids (*6, 23*), whereas α-linolenate is primarily a constituent of the phosphorus-free galactolipids, at least in the phytoflagellates (*22*) and in the green tissues of higher plants (*24*).

Biosynthesis. The ability to continue the desaturation of oleic acid to form multiply unsaturated acids appears to be a property of almost all organisms, the bacteria being the sole exception. Curiously, the introduction of an additional double bond into oleic acid occurs even in those protists and higher plants which fail to carry out the initial desaturation of stearate or palmitate. Mono- and polydesaturations are obviously catalyzed by separate enzymes, even though the mechanisms are grossly very similar, at least with respect to the requirement for oxygen and reduced triphosphopyridine nucleotide. Inspection of existing fatty acid structures suggests that the desaturation of oleate proceeds in two principal directions (Table 4). In one of the sequences (the α-linolenate or "plant" pathway), oleate is progressively desaturated toward the methyl end of the molecule, producing linoleic and α-linolenic acids. In the second (the γ-linolenate or animal pathway), linoleate is also formed initially, but all subsequent double bonds are placed between the 9,10 position and the carboxyl end of the molecule group. As Mead (*25*) and Klenk (*26*) have shown for higher animals, this carboxyl-directed or γ-linolenate pathway leads, in conjunction with chain elongation, to the formation of arachidonate and other C_{20}- and C_{22}-polyunsaturated acids.

In Fig. 1 we present a scheme, adapted from Dougherty and his co-workers (*27*), in which some presumed phylogenetic relationships between groups of existing protists are outlined and patterns of fatty acid biosynthesis which have been observed in these groups are indicated. The relative positions occupied by the organisms do not necessarily imply direct evolutionary descent of one existing group from another; rather, they refer to ancestral and extinct groups

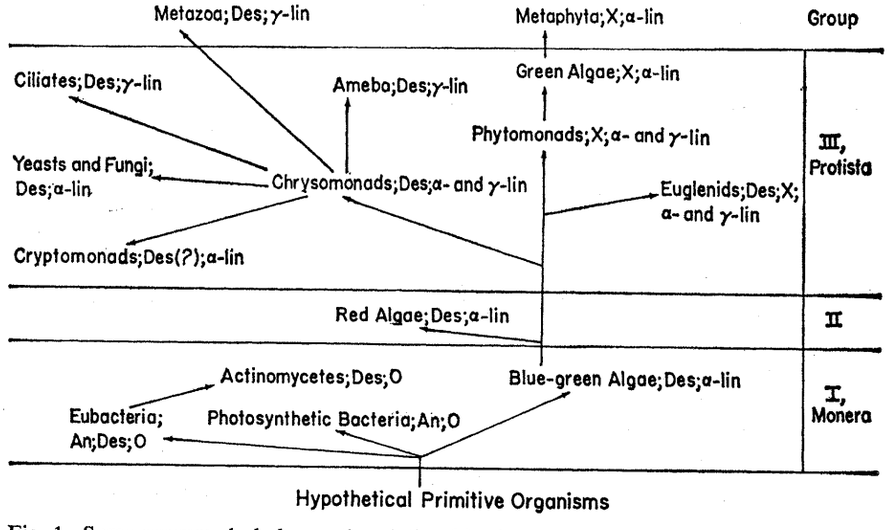

Fig. 1. Some presumed phylogenetic relationships between groups of existing protists, and patterns of fatty acid biosynthesis observed in these groups. *An*, Anaerobic mechanism for the synthesis of monoenoic acids; *Des*, oxidative desaturation for the synthesis of monoenoic acids; *X*, unknown ("plant") mechanism for the synthesis of monoenoic acids; *O*, absence of di- and polyunsaturated fatty acids; *α-lin*, α-linolenate pathway for synthesis of polyunsaturated fatty acids; *γ-lin*, γ-linolenate pathway for synthesis of polyunsaturated fatty acids. Group I, no internal membrane structures, no motor organelles with 2:9 structure; group II, internal membrane structures except mitochondria, no motor organelles with 2:9 structure; group III, internal membrane structures, motor organelles and centrioles with 2:9 structure.

Table 5. Effect of CO_2 concentration, during growth, on properties of chloroplasts of *Euglena gracilis* Z. Cells were grown in the "inorganic" medium of Cramer and Myers (*38*), in either 0.5 or 5 percent CO_2 in air. Chloroplasts were prepared from these cells by the method of Brawermann *et al.* (*39*), and O_2 evolution was measured with benzoquinone as hydrogen acceptor.

Chlorophyll (μg/mg, dry wt)	α-Linolenate (% of total fatty acid)	O_2 evolution in 1.5 hr (μl/mg of chlorophyll)
0.5 Percent CO_2 in air		
22.6	8.4	1) 49.2 2) 60.5
5 Percent CO_2 in air		
28.9	35.0	1) 126.1 2) 166.2

from which current forms were derived. The number of organisms so far examined as representative examples from each group is small, and several important ones, particularly the animal flagellates, have not been studied because of technical difficulties. Hence, the conclusions that follow are necessarily tentative.

As discussed earlier, the chrysomonads are unspecialized metabolically and physiologically and encompass within a single organism all the modes of life found in other groups: phototrophy, heterotrophy, and phagotrophy. The simultaneous operation, in representatives of this group, of the α- and the γ-linolenate pathways for the synthesis of polyunsaturated fatty acids expresses, at the biochemical level, the physiological versatility of these organisms.

From the chrysomonads, three distinct patterns of synthesis of unsaturated fatty acids diverge. In the first group of organisms, represented by the yeasts and fungi, oleate synthesis by oxidative desaturation of stearate and the α-linolenate pathway are retained, while the γ-linolenate pathway is lost, at least above the level of the Phycomycetes. In the second group, comprising the euglenids, the green algae, and the higher plants, the direct desaturation mechanism for monoene formation, and eventually also the γ-linolenate pathway, are lost. In this group of photosynthetic organisms the α-linolenate pathway is retained as the principal synthetic route to polyunsaturated fatty acids.

In the third or animal branch, the amebae, ciliates, and metazoans retain the direct desaturation mechanism for the synthesis of monoenoic acids. Like other protists, the ciliates and amebae convert oleate to linoleate, but the α-linolenate pathway is discontinued, all subsequent desaturations proceeding from the 9,10 position to the carboxyl group of the fatty acid either directly or after further elongation of the fatty acid chain. In ciliates these reactions come to a stop with the synthesis of γ-linolenate (*6*), while in the amebae they continue, leading to synthesis of arachidonate (*14*).

To a limited extent vertebrates further desaturate oleate to form dienoic acids, but this occurs only in the direction of the carboxyl group ($\Delta^{6,9}$ octadecenoate). The formation of the 6,9-dienoic is apparently not useful physiologically because it fails to provide a route to γ-linolenate or arachidonate (*28*). It is for this reason that the $\Delta^{9,12}$ precursors of the fatty acids essential to higher animals must be supplied from dietary sources.

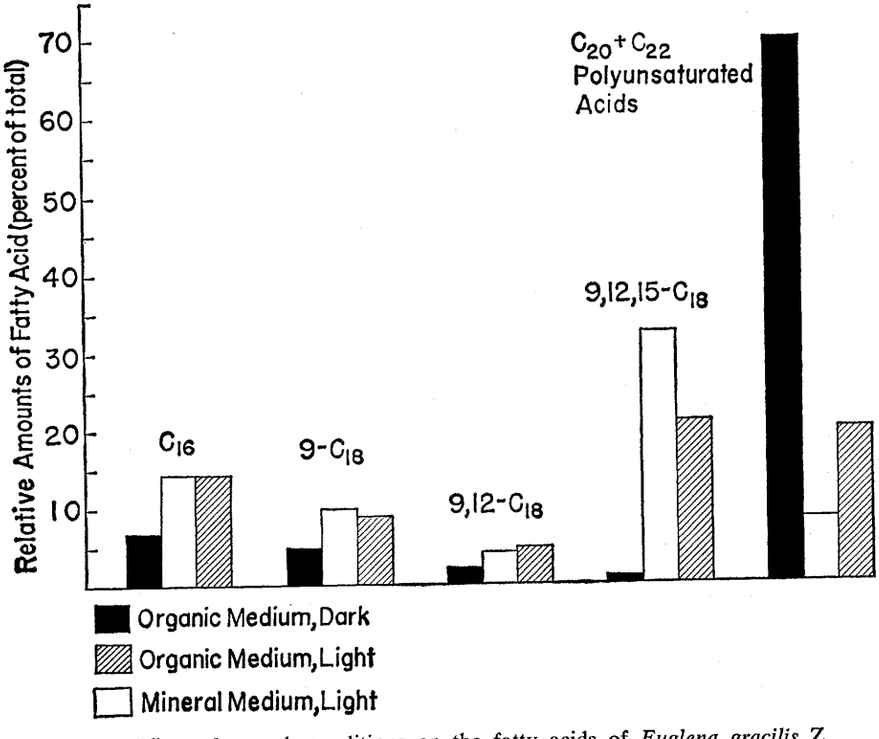

Fig. 2. Effect of growth conditions on the fatty acids of *Euglena gracilis* Z.

Function of Polyenoic Acids

Since the evolutionary trend toward biochemical specialization is reflected in the conservation of the α-linolenate pathway by higher plants and of the γ-linolenate pathway by animals, it may be asked whether the differences in polyenoic acid structure are functionally significant. For studying this question —the correlation between physiological activity and fatty acid (or lipid) structures—*Euglena gracilis* is the organism of choice not only because it has retained the enzyme systems for producing both the fatty acids of the α-linolenate type and those of the γ-linolenate type but also because this phytoflagellate can be grown as a strict auxotroph in the light or, after adaptation, as a heterotroph in the dark. This adaptation is accompanied by a remarkable change in the fatty acid composition of the organism (Fig. 2). When the organism is grown in the light, α-linolenate is the major unsaturated acid of *Euglena,* and the content of C_{20}-polyenoic acids is low. By contrast, when the cells are grown in the dark, the C_{20}-, C_{22}-, and C_{24}-polyenoic acids of the animal type are present in large quantities, while α-linolenate is virtually absent (*22, 29*). Comparison of the lipid patterns of wild type *Euglena* with those of various mutant forms reveals equally striking differences. Artificial mutants of *E. gracilis* which have irreversibly lost the ability to photosynthesize, lack α-linolenate and synthesize the same "animal" type of polyenoic acids as the dark-adapted wild type, even when these mutants are grown in the light. A very similar "animal"-type fatty acid pattern is found in the naturally occurring colorless euglenid *Astasia longa* (*29*). In the permanently or temporarily bleached phytomonads the situation is analogous. They contain little α-linolenate but large amounts of the γ-isomer (*30*). An-

other relevant example is the fatty acid composition of the photosynthetic and nonphotosynthetic forms of some primitive algae. Large amounts of α-linolenate are produced by the blue-green alga *Anabena variabilis* (*7*), whereas *Beggiatoa,* a related but colorless "cyanophyte," synthesizes only mono-unsaturated acids (*30*). The fact that *Beggiatoa* lacks polyunsaturated fatty acids altogether supports, in our view, the classification of this organism as a bacterium. It should be stressed again that in *Euglena* and in higher plants α-linolenate is primarily a constituent of the chloroplast lipids. In *Anabena,* also, α-linolenate is localized in the photosynthetic organelles (chromatophores) (*31*).

The absence of significant quantities of α-linolenate in dark-grown, wild type *Euglena* is not the result of a cessation of α-linolenate synthesis. When this organism is grown in the dark, α-linolenate continues to be formed, to some extent at least, but instead of accumulating, this fatty acid enters an animal-type pathway of polyenoic acid synthesis, in which there is chain elongation and desaturation toward the carboxyl group of the molecule. This modified pathway produces large amounts of a 5,8,11,14, 17,C_{20}-polyenoic acid (*22*). The synthesis of arachidonic acid from linoleic acid is also greatly enhanced. In parallel with this change in fatty acid pattern after adaptation from a photosynthetic to a heterotrophic mode of life, the cellular synthesis of combined lipids also undergoes a marked shift. The galactolipids disappear (*22, 32*), and phospholipids, principally lecithin, are formed instead (*22*). The evidence is therefore strong that the α-linolenate-containing glycolipid formed by *Euglena* in the light is associated with some function of the chloroplast or of some equivalent photosynthetic unit. In support of this view is the finding that not only higher plants and the phytoflagellates but also the primitive blue-green algae synthesize α-linolenate-containing galactolipids (*31*). The only exceptional organisms in this respect are the photosynthetic bacteria. We have already stressed the fact that the photosynthetic bacteria neither synthesize nor require α-linolenate, and it seems significant that in *Chromatium,* one of the organisms examined, galactolipids are absent (*33*). Moreover, the photosynthetic bacteria are unique in that they lack the ability to evolve oxygen during photosynthesis. The results of comparative studies therefore lead to the argument that α-linolenate (and perhaps galactolipid) is a necessary lipid component not for photosynthesis per se but for one or more of the steps that lead to oxygen evolution during photosynthesis. There are several lines of evidence in support of this postulate. For example, a *Scenedesmus* mutant isolated by Bishop, which is defective in the Hill reaction but has morphologically normal chloroplasts (*34*), contains very much less α-linolenate than its parent wild type. Otherwise the fatty acid spectrum of the mutant is normal (*30*). Further evidence suggesting an involvement of α-linolenate in oxygen evolution has been obtained in studies with 3-(*p*-chlorophenyl)-1,1-dimethylurea (or CMU), with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (or DCMU), and with *o*-phenanthroline—substances known to inhibit the Hill reaction. In short-term experiments, light specifically stimulates the incorporation of C^{14}-acetate into α-linolenate by green *Euglena* (*30*). The inhibitors of the Hill reaction abolish this stimulation but do not impair the synthesis of other fatty acids; in fact, the inhibitors of the Hill reaction promote the synthesis of the "animal"-type polyenoic acids (*30*). Light and CMU have similar effects on α-linolenate synthesis in wild type *Scenedesmus,* but not on the mutant that is deficient in the Hill reaction. Hence, it appears that the action of CMU is not to impair desaturation per se but to abolish the increased synthesis of α-linolenate which appears to be associated with the Hill reaction. Finally, the α-linolenate content of green *Euglena* can be shown to depend on other environmental variables that affect photosynthetic evolution of oxygen. The atmospheric concentration of carbon dioxide is one such variable (*35*). *Euglena* cells grown in an atmosphere containing 0.5 percent instead of 5 percent CO_2 yield chloroplasts with an essentially unchanged content of chlorophyll but with a greatly diminished capacity for catalyzing the Hill reaction (with benzoquinone as the hydrogen acceptor). At the same time, the α-linolenate content of "0.5 percent CO_2" chloroplasts is drastically reduced (Table 5). These observations are consistent with the view that α-linolenic acid is essential, either chemically or by virtue of some physicochemical properties, for the operation of O_2-evolving systems in green plants and photosynthetic protists.

The changes in physiological conditions which lead to a decreased α-linolenate content in *Euglena* and other protists are, in general, accompanied by an increase in γ-linolenate, arachidonate, and other longer-chain polyenoic acids. It is therefore tempting to speculate that the polyenoic acids of the "animal" type play a role in the aerobic energy metabolism of heterotrophs that is analogous to whatever role α-linolenate plays in photosynthetic systems. In support of a possible involvement of polyunsaturated fatty acids in oxidative phosphorylation one can cite the "mitochondrial insufficiency" observed in animals raised on diets deficient in essential fatty acids (*36*). Highly suggestive, also, are recent observations showing that glutathione-induced mitochondrial swelling is accompanied by extensive lipid peroxidation (*37*). On the other hand, the fact that many organisms appear to possess functionally perfect mitochondria without containing a high concentration of polyenoic acids of the γ- type—for example, yeasts and insect tissues—makes it more difficult to assess the role of these acids in mitochondrial energy metabolism. It is quite possible, of course, that important differences exist in the efficiency of mitochondrial energy production in various phylogenetic groups.

At any rate, a development of specific and separate functions for polyenoic acids of the α- and γ-types would provide a reasonable explanation of the loss of the γ-linolenate pathway in the evolution of higher plants and of the loss of the α-linolenate route in the evolution of higher animals.

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Comparative Failure in Science

A recent study shows that this is not incompatible with stable careers for basic research scientists.

Barney G. Glaser

A perennial problem for some scientists is their *feeling* of *comparative failure* as scientists. This problem becomes clearer if we consider two major sources of this feeling that are inherent in the very nature of scientific work. (*i*) In science, strong emphasis is placed on the achievement of recognition (*1*); (*ii*) the typical basic scientist works in a community filled with "great men" who have made important and decisive discoveries in their respective fields; they are the acknowledged guiding lights. These esteemed scientists, who have attained honors beyond the reach of most of their colleagues, tend to become models for those who have been trained by them or who have worked under them. As Eiduson has put it in her recent psychological study of basic research scientists (*2*, p. 167): "Scientists are idols-oriented."

To take these honored men as models is important for training as well as for a life in research. During training, one learns to think creatively. Emulation of these models results in the internalization of values, beliefs, and norms of the highest standard. This emulation of the great continues and guides the scientist in his research work, however individual in style his work may be.

But it is precisely here that a feeling of comparative failure may arise. In emulating a great man the scientist tends to compare himself with the model. He estimates how closely he has equaled his model in ability to adhere to high standards of research, to think of relevant problems, to create "elegant" research designs, to devise new methods, to write clearly, to analyze data. In addition, because of the strong emphasis on attaining recognition for research contributions, the scientist perhaps will compare his own degree of success with his model's to gauge how he himself is doing. In using the great man's achievements and the recognition accorded him as criteria, the scientist may be motivated to strive continually and unremittingly toward greater heights (*3*). On the other hand, he may see himself, over time, as a comparative failure for not having attained a comparable amount of recognition (*4*).

Eiduson brings out the dynamics of this problem for scientists (*2*, p. 189): "The model, then, is the ego ideal figure, who represents the ultimate position, and in fact, defines what a scientist should do, how he should think, how he should act. *By comparison, everything else is inevitably of lesser worth* [italics mine]. We have seen the way the scientists in this group rebuke themselves as they become old, distracted, sit on committees or government advisory boards, or become administrators—and thus move away from the ideal. From this picture it is obvious that the scientist is hard on himself. He has a built-in, clearly marked scalar system, along which attitudes and kinds of performances are measured. When he moves away and deviates from the pattern, he becomes a maverick, or a person who has tossed aside the flaming torch."

Average Success

With this problem in mind, I recently made a study of the organizational careers of basic research scientists, one purpose of which was to ascertain the consequences, for the scientist's career, of receiving or not receiving an average amount of recognition (*5*). At the time of the study, these scientists were employed in a government medical research organization devoted to basic research. This was a high-prestige organization from the standpoint of scientists and was run much as though it were a series of university departments. The study is relevant to this discussion in showing something of the career history of basic research scientists, who are today in

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